

Detection of a *bla*_{SHV} Extended-Spectrum β -Lactamase in *Salmonella enterica* Serovar Newport MDR-AmpC

Shelley C. Rankin,^{1*} Jean M. Whichard,² Kevin Joyce,² Lynn Stephens,¹ Kathleen O'Shea,¹
Helen Aceto,¹ Donald S. Munro,¹ and Charles E. Benson¹

Salmonella Reference Center, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, 382 West Street Road, Kennett Square, Pennsylvania 19348-1692,¹ and Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333²

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***Salmonella enterica* serovar Newport MDR-AmpC expressing TEM-1b and extended-spectrum β -lactamase SHV-12 was isolated from affected animals during an outbreak of salmonellosis that led to a 3-month closure of one of the largest equine hospitals in the United States.**

Salmonella enterica serovar Newport MDR-AmpC is undergoing epidemic spread in animals and humans in the United States (4, 5, 7, 18, 22–24). This strain is characterized by a plasmid-mediated *ampC* gene (*bla*_{CMY-2}) that encodes resistance to extended-spectrum cephalosporins (3, 5, 22). Serovar Newport MDR-AmpC strains are commonly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, amoxicillin-clavulanate, cefoxitin, and ceftiofur and show reduced susceptibility to ceftriaxone (23). Serovar Newport MDR-AmpC was isolated from animals between July 2003 and May 2004 during what has now been defined as a protracted outbreak of salmonellosis that subsequently led to a 3-month closure of the George D. Widener Hospital for large animals at the University of Pennsylvania's New Bolton Center (NBC), one of the largest equine hospitals in the United States.

Salmonella strains that express extended-spectrum β -lactamases (ESBLs) are rare. Coexistence of *bla*_{CMY} and ESBL mechanisms in the same *Salmonella* strain has been documented infrequently in the United States and has been associated with foreign travel (8). To our knowledge, this is the first report of ESBL genes in an *S. enterica* serovar Newport MDR-AmpC strain.

All *Salmonella* isolates from the NBC clinical microbiology laboratory that were obtained from July 2003 to May 2004 were referred to the *Salmonella* Reference Center, also at NBC, for serotype confirmation and molecular characterization. Antimicrobial susceptibility profiles of *S. enterica* serovar Newport isolates were determined using a Sensititre CMV2ECOF Companion/Equine MIC veterinary plate (Trek Diagnostics, Cleveland, Ohio), and the interpretation of breakpoints was as determined by the manufacturer according to CLSI (formerly NCCLS) guidelines, when available (14–16). Serovar Newport strain SRC0307-213, isolated in July 2003 and identified as the index case, was shown to be resistant to ampicillin, chloramphenicol, tetracycline, cephalothin, ceftiofur, amoxicillin-clavulanate, gentamicin, and trimethoprim-

sulfamethoxazole. Isolates from all of the 60 *S. enterica* serovar Newport cases included in the present report were susceptible to amikacin, imipenem, and enrofloxacin.

Plasmids were transferred by conjugation from *S. enterica* serovar Newport strain SRC0307-213 to a nalidixic acid-resistant *Escherichia coli* recipient strain (ATCC 27662) using nalidixic acid (50 μ g/ml) and ampicillin (50 μ g/ml) as the selective agents. Isoelectric focusing (IEF) for β -lactamases was performed on all strains at the Centers for Disease Control and Prevention (CDC) using a small-scale freeze-thaw method (12). Detection of *bla*_{CMY} and *bla*_{TEM} genes was performed by PCR at NBC as described previously (18). Detection of *bla*_{SHV} was performed at CDC using a modification of the method described by Rasheed and colleagues (19). Briefly, the *bla*_{SHV} gene was amplified on a PTC-200 DNA engine (MJResearch, Waltham, MA) in a final volume of 25 μ l. A final primer concentration of 0.2 μ M was used, and cycling conditions were as follows: 96°C for 5 min, 35 cycles of 96°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min.

β -Lactamase genes were sequenced using standard methods. Briefly, total genomic DNA was extracted from SRC0307-213 and *E. coli* transconjugants using a Wizard genomic DNA purification kit (Promega Corp., Madison, WI). β -Lactamase genes were amplified with Promega 2 \times PCR master mix using primers shown in Table 1. PCR amplicons were cleaned up using a QiaQuick PCR purification kit (QIAGEN, Valencia, CA). Clean amplicons were used to obtain 2 \times DNA sequence coverage in both directions using a CEQ2000 capillary sequencer (Beckman Coulter, Fullerton, CA), and sequence alignments were performed using SeqManII version 5.06 (DNASTar Inc., Madison, WI).

Salmonella enterica serovar Newport SRC0307-213 was phenotypically negative for ESBL production by double-disk diffusion testing with ceftazidime and ceftazidime-clavulanate and also cefotaxime and cefotaxime-clavulanate (BD BBL Sensi-Disk; Becton Dickinson, Franklin Lakes, NJ) but was positive for *bla*_{CMY}, *bla*_{TEM}, and *bla*_{SHV} genes by PCR. It has been noted previously that the presence of an ESBL can be masked by the expression of an AmpC-like enzyme, such as *bla*_{CMY} (1, 2, 11). IEF showed enzymes with pIs of \geq 8.4, 8.0, and 5.4, consistent with the CMY, SHV, and TEM enzymes, respectively. Two *E. coli* transconju-

* Corresponding author. Mailing address: *Salmonella* Reference Center, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348-1692. Phone: (610) 925-6596. Fax: (610) 925-8115. E-mail: srankin@vet.upenn.edu.

TABLE 1. Oligonucleotide primers used to amplify genes for sequencing

Gene target	Primer	Sequence	Product size (bp)
<i>bla</i> _{CMY}	CMY-F	5'-CACACGTGGAATTTAGGAAAA-3'	1,384
	CMY-R	5'-CAAAATGCGCATGGGATT-3'	
<i>bla</i> _{SHV}	SHV-F	5'-GGTTATTCTTATTGTGCTTCTT-3'	1,233
	SHV-R	5'-TACGTTACGCCACCTGGCTA-3'	
<i>bla</i> _{TEM}	TEM-F	5'-TGGCACTTTTCGGGGAAAT-3'	1,127
	TEM-R	5'-ACGCTCAGTGGAACGAAAACT-3'	

gants (SRC0307-213-1 and SRC0307-213-2) were tested by double-disk diffusion as described above, and both exhibited an ESBL phenotype. Both transconjugant strains were *bla*_{TEM} and *bla*_{SHV} positive and negative for the *bla*_{CMY} gene by PCR. IEF of the transconjugants showed that only two β -lactamase genes, encoding enzymes with pIs of 5.4 and 8.0, had transferred, and this was consistent with the fact that the plasmid-mediated *ampC* gene in serovar Newport strains has previously been shown to be difficult to transfer by conjugation (6). DNA sequencing of *S. enterica* serovar Newport strain SRC0307-213 showed that it contained a *bla*_{CMY-2} gene. *E. coli* transconjugant EC0307-213-2 was positive for *bla*_{TEM-1b} and the extended-spectrum β -lactamase gene *bla*_{SHV-12}.

ESBL-producing salmonellae are rare in the United States, and this is the first report of an ESBL-producing *S. enterica* serovar Newport MDR-AmpC strain from animals. *bla*_{TEM-1b} has frequently been found in *Enterobacteriaceae*, including *Salmonella* (10), and the *bla*_{SHV-12} gene, first described for *E. coli* and *Klebsiella pneumoniae* from Switzerland in 1997, is now becoming increasingly common in *Salmonella* serotypes from Europe and the United Kingdom but has not yet been described for *S. enterica* serovar Newport (9, 13, 17, 20, 21). Isoelectric focusing of β -lactamases characterized so far from human *S. enterica* serovar Newport MDR-AmpC strains submitted to the National Antimicrobial Resistance Monitoring System indicate that many express a putative TEM enzyme, but none show enzymes in the range expected for SHV β -lactamases (data not shown). The identification of ESBL genes in *S. enterica* serovar Newport MDR-AmpC has considerable implications for veterinary and public health. Carriage of multiple β -lactamase genes is disconcerting because certain combinations of genes could effectively limit all β -lactam therapeutic options.

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